REMARKS

Status

Claims 1-3, 8, 10, 12, 13, 21, 30, 31 and 68 as set forth in the preliminary amendment which accompanied the filing of this patent application were at issue in this present Office Action.

The present response does not cancel any claim, and adds new claim 71. Accordingly, it is now claims 1-3, 8, 10, 12, 13, 21, 30, 31, 68 and 71, as amended, which are at issue.

The Office Action

In the Office Action mailed November 2, 2007, all claims then pending were rejected. Specifically, claims 1-3, 8, 10, 12, 13, 21, 30, 31 and 68 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for reasons as specifically noted by the Examiner.

Claims 1, 2, 8, 10, 30-31 and 68 were rejected under 35 U.S.C. §102 as being anticipated by U.S. Patent 4,649,114 of Miltenburger.

Claims 1-3, 8, 10, 21, 30, 31 and 68 were rejected under 35 U.S.C. §102 as being anticipated by the journal article of Wodnicka.

Claims 1-3, 8, 10, 12, 13, 21, 30, 31 and 68 were rejected under 35 U.S.C. §102 as being anticipated by the journal article of Houghton.

Claims 1-3, 8, 10, 30, 31 and 68 were rejected under 35 U.S.C. §102 as being anticipated by Trimarchi.

Claims 1-3, 8, 10, 21, 30, 31 and 68 were rejected under 35 U.S.C. §102 as being anticipated by EP 1,134,583.

Claims 1-3, 8, 10, 12, 13, 21, 30, 31 and 68 were rejected under 35 U.S.C. §103 as being unpatentable over U.S. Patent 5,763,279 of Schwarz taken in view of Miltenburger '114.

Claims 1-3, 8, 10, 21, 30, 31 and 68 were rejected under 35 U.S.C. §103 as being unpatentable over published U.S. Application 2002/0072113 of Barbera-Guillem taken in view of the journal article of Jung et al.

Applicant thanks the Examiner for the search, for the Office Action, and for the thorough explanation of the basis of the rejections.

The Present Invention

Applicant will briefly recapitulate the principles of the present invention so as to better differentiate it from the prior art. The present invention is directed to a system and method for measuring the metabolic rate of a single, individual, substantially spherical particle. The particle is disposed in a medium which is contained within a compartment which is defined by a diffusion barrier. The diffusion barrier further operates to restrict and reduce the diffusive flux of metabolites to and from the particle and in that regard may be permeable or may include one or more openings or passages therethrough. The diffusion barrier functions to sustain a metabolite diffusion gradient in the medium, and a detector measures the concentration of the metabolite inside the compartment so as to allow for the measurement of the metabolic rate of the individual particle.

More particularly, the device according to the present invention provides a diffusion barrier, which defines a compartment. In that compartment is contained a medium with a metabolizing particle. The distinguishing feature of the device compared to the prior art is that this diffusion barrier allows a metabolite diffusion gradient to be established from the metabolizing particle and throughout the medium disposed in the compartment. The diffusion barrier can take different forms. In some embodiments, it comprises a gas permeable lid that separates the medium in the compartment from the surroundings. An example of such a device

is seen in Figure 8. In other embodiments, the diffusion barrier is defined by a geometrical constriction, where the diffusive flux of the metabolites through the medium within this constriction is reduced and a metabolite diffusion gradient can be established. Such a geometrical constriction can be realized for instance by positioning impermeable structures close to each other with only a narrow gap separating these structures. Some examples of embodiments, wherein the diffusion barrier is realized in this manner, are illustrated in Figure 9 and Figure 10. In Figure 9, the upper part of the compartment wall is constituted by a disk of a substantially impermeable material. The lower part of the compartment wall is then substantially constituted by the area of the bottom of the container covered by the disk. In yet another embodiment of the device according to the present invention, a narrow opening in the diffusion barrier provides means for diffusion of metabolites to and from the metabolizing particle. Some examples of such embodiments are seen in Figures 12 and 13.

The diffusion barrier that defines a compartment distinguishes the device from the devices according to the cited prior art references, since none of these disclose such a device. In contrast, the cited prior art references describe open devices, such as the Petri dishes used by Trimarchi et al. In such open devices, a diffusion gradient can only be established in the immediate vicinity of a metabolizing particle. It will only be possible to establish a metabolite diffusion gradient throughout the medium in a sample compartment, when the compartment is defined by a diffusion barrier.

The Amended Claims

In this response, independent claim 1 has been amended to emphasize that it is the metabolic rate of an individual particle that is measured within the device and not the individual metabolic rate of metabolizing particles. The claim has been further amended to emphasize that

a metabolite diffusion gradient is established throughout the medium retained in the compartment and that the transport of metabolites to and from the metabolizing particle, through the medium, occurs through diffusion. Claim 1 has been further amended to replace "comprising" with "retaining" in line 10 to avoid any confusion and make clear that the compartment retains the medium.

Claim 2 has been amended to exclude the phrase "and the medium" in response to the Examiner's clarity objections under 35 U.S.C. §112, second paragraph.

Claim 3 has been amended to correct the antecedent basis for the "at least one compartment wall" referred to in claim 2. Claim 8 has been amended to give a range for the recited viscosity thereby overcoming the rejection under 35 U.S.C. §112, second paragraph.

Claim 68 has been amended to include the same limitations as in claim 1.

New claim 71 has been added. This claim corresponds to claim 17 of the original PCT application.

The Rejections under 35 U.S.C. §112

In view of the amendments submitted herewith, all claims now overcome the rejections under 35 U.S.C. §112, second paragraph.

The Rejections under 35 U.S.C. §102

I. Miltenburger et al. (US 4,649,114)

Miltenburger et al. describes a fermentation apparatus, which operates to achieve a high concentration of metabolites in a fermentation vessel, while avoiding air bubbles. Metabolites are hence provided to the medium through a gas permeable membrane. In order to improve the growth conditions in the fermentation vessel, the metabolites should preferably be distributed homogeneously throughout the medium in the vessel. The device according to Miltenburger is

therefore equipped with propelling means to ensure an efficient mixing of the metabolite in the medium and an efficient transport of the metabolites to the cells. In col. 2 line 64 to col. 3, line 1 is specifically described that each volume on the microliter scale will contain the **same amount of oxygen**. The transport of the metabolites in the medium is not governed by diffusion but by flow induced mixing and Miltenburger et al. does hence explicitly disclose a device, wherein **no metabolite diffusion gradient is established** throughout the medium in the compartment.

Means for measuring the amount of oxygen inside the vessel are provided, but there is no disclosure of a system wherein the metabolic rate from individual particles is measured non-invasively.

Given that the independent claims of the present invention relates to a device, wherein a metabolite diffusion gradient is established throughout the medium in the compartment, the independent claims and all dependent claims of the present invention are novel in view of Miltenburger et al.

II. Wodnicka (J. Biomolecular screening, 2000)

This reference describes a fluorescence based method for monitoring the number and viability of a **large number** of cells positioned in a microplate well (>1500 cells/well; p. 142, left col., line 14 from below). The optical detection of the cells present in the microtiter plate wells in the device according to Wodnicka et al. is based on an oxygen sensitive fluorescent compound that is embedded in a silicone rubber (p. 142, right col., lines 9-1 from below) that is attached to the bottom of the microplate wells. Since the oxygen level is correlated to the number and viability of the cells, the fluorescence signal detected from the well provides information about the number of cells in that well and their viability. In Figure 5B and the accompanying text is illustrated that the method according to Wodnicka et al. is suitable for

determining the number of cells in a medium, when this number is in the range of several thousands to hundreds of thousands. There is however no disclosure of a method for evaluating the metabolic rate of individual particles. In fact, Wodnicka et al. inherently assumes that all particles have the same metabolic rate in order to being able to link the fluorescence from one well to the number of particles inside this well.

Wodnicka et al. arrange the particles and the medium in microtiter plate wells (e.g. Falcon U-bottom 96-well microtiter plates or 24-well tissue culture plates, page 142, right col., lines 5-7) with a diameter in the order of several millimetres. The turbulence caused by the convective currents present in wells of such a diameter will result in **a mixing of the medium, whereby a metabolite diffusion gradient is not allowed** to be established throughout the medium even when no means for mixing the medium are provided. In order to establish a metabolite gradient throughout the medium in the microtiter plate wells used by Wodnicka et al., the diffusive flow of the metabolites must be restricted by a diffusion barrier such as a lid (e.g. Figure 8 of the current application) or a constriction, such as the construction according to Figure 11 of the current application (described on page 62, lines 7-33 of current application) or the constriction according to Figure 2. However, there is no disclosure of such devices in Wodnicka et al.

The several thousand cells present in each well in the method according to Wodnicka et al. deposits on top of the layer of silica rubber containing the oxygen sensitive fluorescent compound, and hence takes the form of a substantially cylindrically shaped distribution. The Examiner's comments concerning the particle-like structure of the distribution of cells in the well stating that such a particle is substantially spherical is hence by no means correct.

The sensitivity of the device according to the present invention is 3 orders of magnitude better than that of the device according to Wodnicka et al., which simply **does not provide means for measuring the metabolite rate of individual particles**. In contrast, the device according to the present invention provides means for measuring the metabolic rate of a single particle. There is furthermore absolutely no disclosure in Wodnicka et al. of a device wherein a metabolite diffusion gradient is established throughout the medium and where the metabolic rate of individual substantially spherical particles is determined. The independent claims and all dependent claims of the present invention are hence novel in view of Wodnicka et al.

III. Houghton et al. (Mol. Reprod. and Dev.)

In this reference, a medium comprising metabolizing particles is positioned inside a PCR micropipette together with an amount of oxygen saturated pyrene, a luminophore whose fluorescence is quenched by oxygen. The device is then sealed at both ends, one end with sealing wax, and the other end with a clamp (p. 477, right col., lines 18-20 and Figure 1). Due to the metabolism of the metabolizing particles, and the air tight seals provided at the ends of the micropipette, an oxygen concentration gradient will then be established (p. 478, left col., lines 3-5).

However, since the micropipette is air tightly sealed, Houghton et al. **does not disclose a device, wherein metabolite transport through a diffusion barrier is allowed by diffusion**. The independent claims and all claims dependent thereon (including claims 13 and 21) are hence novel in view of Houghton et al.

IV. Trimarchi et al. (Bio. of Reprod.)

Trimarchi et al. describes a method wherein the metabolite concentration gradient in the vicinity of a metabolizing particle is measured. The metabolizing particle is arranged in an open Petri dish and the oxygen gradient is measured using an oxygen electrode. However, Trimarchi et

al. does not disclose a method, wherein a diffusion gradient is established throughout the medium in the compartment. As described in the Discussion (p. 1872, left col., lines 19-22), the oxygen concentration is only influenced some 50 micrometers away from the metabolizing particle.

V. EP 1 134 583

This reference describes a method for measuring metabolic rate changes of organisms by repeatedly or continually measuring the concentration of a metabolic gas in a container housing the organism in order to determine changes in consumption or production of a metabolic gas. The container of EP 1 134 583 does not comprise a diffusion barrier that allows diffusion of metabolites to diffuse into the container. **The device according to EP 1 134 583 does hence not allow a metabolite diffusion gradient to be established throughout the medium in accordance with the present invention.** There is furthermore no description of how a controlled diffusion of gas to or from the particle should be conducted. Metabolites may be provided to the medium in the container, but this happens through a valve or an injection system (page 5, lines 7-8).

There is hence no disclosure in EP 1 134 583 of a device, wherein metabolite transport through a diffusion barrier to and from the particle is allowed by diffusion, whereby a metabolite diffusion gradient is established throughout the medium.

The device according to independent claims 1 and 68 of the present application are hence novel in view of EP 1 134 583.

The Rejections under 35 U.S.C. §103

I. Miltenburger et al. (US 4,649,114)

As described above, Miltenburger et al. shows a fermentation apparatus, wherein metabolites provided through a gas permeable membrane to a medium in the fermentation vessel,

are distributed homogenously in the medium due to the action of propelling means. **A metabolite diffusion gradient is hence not established** throughout the medium. Miltenburger et al. does mention the possibility of changing the shape of the diffusion barrier, but such a modification does not change the fact that the metabolites introduced into the medium through the permeable material are distributed homogeneously by the propelling means. Miltenburger et al. does not provide any hint at all to a device wherein a metabolite diffusion gradient is established throughout the medium. Instead in col. 2 line 64 to col. 3, line 1, Miltenburger et al. explicitly describes that each volume on the microliter scale will contain the same amount of oxygen. This reference thus evidently teaches away from establishing such a gradient throughout the medium in the compartment, and the independent claims and all dependent claims of the present invention are hence non-obvious in view of Miltenburger et al.

II. Schwartz et al. (US 5,763,279) in View of Miltenburger et al. (US 4,649,114)

Schwartz et al. describes a bioreactor for cell culture. The bioreactor comprises a diffusion barrier that allows metabolites to diffuse in and out of the compartment defined by the diffusion barrier. The metabolites are held suspended in the medium by clinostatic suspension, where the compartment is rotated about its horizontal longitudinal axis (col. 2, lines 19-20, col. 3, lines 31-32). Thereby the metabolites are kept suspended in the medium with a minimum of turbulent mixing. The bioreactor operates to maximize cell growth, and thus mixes the culture medium, by rotation of the vessel, to assure full oxygenation. No oxygen gradient is established. The bioreactor provides no means for measuring the concentration of a metabolite and provides no hints to including such means.

These features are in clear contrast to the features of the device according to Miltenburger et al., where thorough mixing is provided by propelling means to obtain a homogeneous

distribution of the metabolites in the medium. The devices according to Schwartz et al. and Miltenburger et al. hence utilize completely different and incompatible concepts for entering the metabolites into the medium in the vessel/bioreactor. The person skilled in the art would hence never contemplate to realize the device according to the present invention from the combined teachings of these references with a reasonable expectation of success.

As the devices of Miltenburger et al. and Schwartz et al. comprises a number of clearly distinct features, the device according to the current application can only be constructed from the combined teachings of these references by using impermissible hindsight.

In conclusion, the device according to the present invention is non-obvious in view of Miltenburger et al. combined with Schwartz et al.

III. Barbera-Guillem et al. (US 2002/0072113) in View of Jung et al. (Anal. Chem., Sept. 1999)

Barbera-Guillem et al. describes a device for heating cultured cells and maintaining a desired temperature in connection with the high-volume culturing of cells. The compartment comprises a permeable part through which providing an **optimal** transfer of gasses to the medium, wherein the cells are cultured (page 2, right col., lines 37-39). The heating element can for instance be provided as a thin layer attached to the compartment. The device may comprise a port that allows a tip to enter the compartment (page 2, right col., lines 11-24). Barbera-Guillem hence is concerned with the delivery of a large volume of gas to a thin film layer of culture medium, and not with establishing a metabolic gradient with regard to an individual, isolated particle.

Jung et al. presents an oxygen sensor using a micrometer sized tip for monitoring the oxygen gradient around single cells mouse pancreatic islets. As seen in Figure 3, the device is

capable of recording the oxygen content at various distances from the surface of mouse pancreatic islets.

From the description of the permeable membrane providing means for an optimal transfer of gas into the compartment, it is clear that this does not constitute the diffusion barrier according to the independent claims of the current application. The teachings of Barbera-Guillem et al. and Jung et al. can hence not be combined in a manner, wherein the device according to the current application is disclosed.

Conclusion

In view of the amendments and remarks presented herein, Applicant respectfully submits that all claims are now clearly differentiated over the prior art of record taken either singly or in combination. Furthermore, all rejections under 35 U.S.C. §112 are overcome. The application is in condition for allowance. Any questions, comments or suggestions which the Examiner may have, which will place the application in still better condition for allowance, should be directed to the undersigned attorney.

Dated:

Respectfully submitted,

By_____

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